Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-2447

Research Article

Xenobiotic Metabolizing Gene Variants, Dietary Heterocyclic Amine Intake, and Risk of Prostate Cancer

Stella Koutros,[1] Sonja I. Berndt,[1] Rashmi Sinha,[1] Xiaomei Ma,[2] Nilanjan Chatterjee,[1] Michael C.R. Alavanja,[1] Tongzhang Zheng,[2] Wen-Yi Huang,[1] Richard B. Hayes,[1] and Amanda J. Cross[1]

[1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Department of Health and Human Services, Rockville, Maryland and [2]Department of Epidemiology and Public Health, Yale School of Medicine, New Haven, Connecticut

Abstract

We recently reported that heterocyclic amines (HCA) are associated with prostate cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. We now use extensive genetic data from this resource to determine if risks associated with dietary HCAs {2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP); 2-amino-3,8-dimethylimidazo[4,5-*b*]quinoxaline (MeIQx); and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx)} from cooked meat are modified by single nucleotide polymorphisms (SNP) in genes involved in HCA metabolism (*CYP1A1, CYP1A2, CYP1B1, GSTA1, GSTM1, GSTM3, GSTP1, NAT1, NAT2, SULT1A1, SULT1A2*, and *UGT1A* locus). We conducted a nested case-control study that included 1,126 prostate cancer cases and 1,127 controls selected for a genome-wide association study for prostate cancer. Unconditional logistic regression was used to estimate odds ratios (OR), 95% confidence intervals (95% CI), and *P* values for the interaction between SNPs, HCA intake, and risk of prostate cancer. The strongest evidence for an interaction was noted between DiMeIQx and MeIQx and the polymorphism rs11102001 downstream of the *GSTM3* locus ($P_{interaction}$ = 0.001 for both HCAs; statistically significant after correction for multiple testing). Among men carrying the *A* variant, the risk of prostate cancer associated with high DiMeIQx intake was 2-fold greater than that with low intake (OR, 2.3; 95% CI, 1.2–4.7). The SNP rs11102001, which encodes a nonsynonymous amino acid change *P356S* in *EPS8L3*, is a potential candidate modifier of the effect of HCAs on prostate cancer risk. The observed effect provides evidence to support the hypothesis that HCAs may act as promoters of malignant transformation by altering mitogenic signaling. [Cancer Res 2009;69(5):1877–84]

Introduction

Recent studies suggest that exposure to heterocyclic amines (HCA) derived from meats cooked at high temperatures, such as pan-frying or barbequing, may increase the risk of prostate cancer. Several animal and human experimental studies have shown the carcinogenicity of three HCAs in particular: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP); 2-amino-3,8-dimethylimidazo[4,5-*b*]quinoxaline (MeIQx); and 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx). In animal models, PhIP increases mutation frequency (1) and tumor incidence (2) in the prostate. *In vitro* work in human prostate cells has shown that PhIP increases genotoxicity and DNA adduct levels (3–5); PhIP-DNA adducts have been also been detected *in vivo* in human prostate cells (6, 7). Oral administration of MeIQx induces tumors in rodents at multiple tissue sites (8). The *N*-hydroxy metabolite of MeIQx leads to prostate hyperplasia in rats and induces MeIQx-DNA adduct formation in human prostate epithelial cells (5, 9). DiMeIQx, which is similar in chemical structure to MeIQx, is mutagenic in bacterial assays (10) but has not been extensively evaluated as an animal or human carcinogen.

Epidemiologic studies of HCA intake and prostate cancer are limited. One large prospective study found a significant increased risk of prostate cancer for individuals in the highest quintile of PhIP intake (11), whereas another prospective study found elevated risks associated with increased MeIQx and DiMeIQx (12). Two small case-control studies, however, found no association between these HCAs and prostate cancer (13, 14).

Once HCAs enter the body, they undergo a series of chemical reactions in order to be eliminated. These reactions are highly dependent on particular xenobiotic metabolic enzymes (XME) and include both phase I and phase II enzymes. The metabolism of MeIQx, the structurally similar DiMeIQx, and PhIP has been extensively described (15). Cytochrome *P*450 (CYP) enzymes (phase I) including *CYP1A1, CYP1A2*, and *CYP1B1* are involved in the bioactivation of these compounds. Phase II enzymes including sulfotransferases (*SULT*), *N*-acetyltransferases (*NAT*), UDP-glucuronosyltransferases (*UGT*), and glutathione *S*-transferases (*GST*) are responsible for further metabolism and detoxification. Single nucleotide polymorphisms (SNP) in genes that code for these enzymes may result in differential metabolism of HCAs and their intermediates and, thus, may be related to prostate cancer risk. SNPs in genes related to xenobiotic metabolism have been inconsistently associated with prostate cancer risk (16–19). The conflicting findings may be a result of an oversimplification in assuming that these genes act alone to alter risk. The complex interaction with environmental exposures, like those from dietary HCA intake, may offer more insight into the importance of XMEs and prostate cancer risk.

By combining data from a genome-wide association study of prostate cancer and dietary HCA intake from questionnaire data, we evaluated the interaction of dietary HCA (PhIP, MeIQx, and DiMeIQx) intake, polymorphisms in genes involved in HCA metabolism (*CYP1A1, CYP1A2, CYP1B1, GSTA1, GSTM1, GSTM3, GSTP1, NAT1, NAT2, SULT1A1, SULT1A2*, and *UGT1A* locus), and risk of prostate cancer.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Requests for reprints: Stella Koutros, Occupational and Environmental Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Boulevard, EPS 8111, MSC 7240, Rockville, MD 20852. Phone: 301-594-6352; Fax: 301-402-1819; E-mail: KoutrosS@mail.nih.gov.

doi:10.1158/0008-5472.CAN-08-2447

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-2447

Materials and Methods

Study population. Participants were selected from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, a randomized, controlled, multisite trial to test the efficacy of screening methods for these four cancers. The details of this trial have been described elsewhere (20, 21). Briefly, the PLCO trial participants were individuals 55 to 74 y old, who were enrolled between 1993 and 2001, and reported no history of prostate, lung, colorectal, or ovarian cancer. Participants were randomized to either the screening or control arm of the trial. Men randomized to the screening arm were offered a prostate-specific antigen (PSA) test and digital rectal exam at baseline and annually thereafter for 3 y, followed by 2 y of screening with PSA alone. Cancer diagnoses were ascertained from mailed annual questionnaires and supplemented by trial screening results and linkage to cancer registries and the National Death Index to enhance completeness. Cases of prostate cancer were confirmed by review of medical records. The study protocol was approved by the institutional review board at each study center and the National Cancer Institute, and participants provided informed consent.

A subgroup of men from the screening arm of the PLCO trial were included in the National Cancer Institute genome-wide association study of prostate cancer called Cancer Genetics Markers of Susceptibility (CGEMS), an initiative that has genotyped ~550,000 SNPs in selected prostate cancer cases and controls.[3] Details of the CGEMS case and control selection are described elsewhere (22). In brief, cases and controls were Caucasian, completed the baseline questionnaire, provided a blood sample, had no prior history of prostate cancer before randomization, and had at least one PSA test before October 1, 2003. Cases were oversampled for aggressive disease (Gleason score ≥7 or stage ≥III) and were diagnosed between October 1993 and September 2003. A total of 1,230 prostate cancer cases were selected. Controls were selected by incidence density sampling resulting in the identification of 1,204 different men and 1,230 control selections (1,179 subjects sampled once, 24 subjects sampled twice, and 1 subject sampled thrice). Cases and controls were frequency matched on age at randomization, fiscal year of randomization, and time since initial screen.

Additional eligibility criteria for the current nested case-control study follow those of the previous meat and meat-mutagen analysis from the PLCO cohort (11). Cases and controls were ineligible if they did not complete the PLCO food frequency questionnaire (FFQ; $n = 148$), were extreme outliers for reported energy intake (those in the top or bottom 1% of intake; $n = 37$), were missing information on HCAs ($n = 1$), or missed more than seven items on their FFQ ($n = 21$), resulting in a total sample size of 2,253 (1,126 cases and 1,127 controls).

HCA assessment. Dietary data were collected as part of the PLCO trial from a validated 137-item FFQ at baseline. The FFQ included detailed information about meat-cooking methods (barbequing, grilling, pan-frying, and broiling) and extent of doneness (rare, medium, well done, or very well done) for commonly consumed meats (steak, bacon, sausage, pork chop, and hamburgers) often cooked by different methods. Information obtained from the FFQ was used in conjunction with a mutagen database, Computerized Heterocyclic Amines Resource for Research in Epidemiology of Diseases (CHARRED; ref. 23), to estimate intake of PhIP, MeIQx, and DiMeIQx.

Genotyping and gene selection. Selection of SNPs, genotyping, and quality control procedures for the CGEMS prostate cancer study is described in detail in the Supplementary Methods of the article by Yeager and colleagues (22), which can be found online.[4] Briefly, genotyping of the CGEMS prostate cancer study was done under contract by Illumina Inc. using the HumanHap240 and HumanHap300 platforms, which constituted a fixed panel of 561,494 tagSNPs. Common (minor allele frequency, ≥5%) SNPs were identified using the method described by Carlson and colleagues (24), assuming a r^2 threshold of >0.8 for the HapMap CEU subjects of European ancestry. For the European population, this panel is expected to cover close to 90% of the common SNPs in HapMap phase 1. Genotype data for all SNPs in the current study were tested among controls for possible deviation from Hardy-Weinberg proportions.

We selected genes in phase I and phase II enzymes that are directly involved in the metabolism of the HCAs under study (15, 25). The following 10 genes or gene regions (combined due to proximity and overlapping regions) were included in the current study: *CYP1A1* and *CYP1A2* region; *CYP1B1, GSTA1, GSTM1, GSTM3, GSTP1, NAT1, NAT2, SULT1A1*, and *SULT1A2* region; and the *UGT1A* locus. For each gene or gene region, a margin spanning 20 kb upstream and 10 kb downstream was used to select SNPs.

A total of 127 SNPs were selected for analysis; eight SNPs (rs13406898, rs17038616, rs17864673, rs17868306, rs7192559, rs8191431, rs9341252, and rs9939264) showed little or no variation (minor allele frequency, <1%) and thus were not included in regression modeling. All other SNPs had a minor allele frequency of ≥1% in our population.

Statistical analysis. Differences between cases and controls with respect to descriptive characteristics were assessed using a χ^2 test for categorical variables and t test for continuous variables. Unconditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for the association between HCA intake and prostate cancer; the association between XME SNPs and prostate cancer (data not shown); and the interaction between SNPs in XME genes, HCA intake, and prostate cancer. SNP-HCA interactions were examined using a multiplicative model. Interactions were assessed by including the cross-product terms for the gene (SNP) and HCA intake as well as the main effect terms in a logistic regression model. Genotypes were categorized using ordinal values of 1 (homozygote wild-type), 2 (heterozygote), and 3 (homozygote variant) assuming an additive model in the regression. We evaluated the interaction under different genetic models (codominant, dominant, and recessive); however, these results were similar to those presented assuming an additive model and are therefore not shown. In instances in which the homozygous variant genotype was rare, this was combined with the heterozygous group. HCA intake was categorized as low (0–39th percentile), medium (40–79th percentile), and high (≥80th percentile) intake because the intake distribution is skewed and previous analyses have identified the top quintile as potentially the most important (12). Models were adjusted for age at selection, study center, and total energy intake. The P value for each SNP-HCA interaction was computed by comparing nested models with and without the cross-product terms using a likelihood ratio test. Interactions with $P \leq 0.15$ are presented.

Given the small P values, the presence of functional variants, and the consistent indication of interaction between these genes and HCAs, associations between HCA intake and prostate cancer stratified by *GSTM3* and *GSTP1* genotypes were further evaluated for risk patterns. The association between joint categories of HCA intake and the same SNPs was also estimated; the reference group was defined as the combination of the homozygote wild-type genotype and the lowest HCA intake group. Statistical analyses were done using STATA version 9.0.

Haplotype-HCA interaction analyses assuming an additive model for each haplotype were pursued for *GSTM3* and *GSTP1*; haplotype-HCA interactions were explored for the *GSTP1* locus with PhIP and MeIQx and for *GSTM3* with all HCAs. Haplotypes were estimated using the expectation-maximization algorithm (26) in HaploStats version 1.2.1 for the R-programming language. Haplotypes with a frequency of <1% were collapsed into a single category and the most common haplotype was used as the reference. The P value for each haplotype-HCA interaction was computed by comparing nested models with and without the cross-product terms using a likelihood ratio test.

To test the robustness of our findings, the false discovery rate (FDR; Benjamini-Hochberg adjustment) method was applied (27). The FDR is the expected proportion of false discoveries among the discoveries. FDR values were calculated separately for each HCA from the results of 119 tests (i.e., total number of SNPs studied minus those with no variation) evaluating the association between each SNP-HCA interaction and the risk of prostate cancer. Interactions were deemed significant at the FDR = 0.20 level; this indicates that one in five discoveries would be expected to be false.

[3] http://cgems.cancer.gov/data/

[4] http://www.nature.com/ng/journal/v39/n5/suppinfo/ng2022_S1.html

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-2447

Table 1. Characteristics of prostate cancer cases and controls

Characteristic	Cases (N = 1,127)	Controls (N = 1,126)	P*
Age at randomization (n, %)			
55–59	125 (11.1)	131 (11.6)	
60–64	258 (22.9)	275 (24.4)	
65–69	360 (31.9)	368 (32.7)	
70–74	384 (34.1)	352 (31.3)	0.54
Extent of disease†			
Nonaggressive	473 (42.0)	—	
Aggressive	654 (58.0)	—	
HCA intake			
PhIP (ng/d)			
Median (IQR)	80.0 (34.6–212.2)	86.6 (36.1–218.0)	0.44
MeIQx (ng/d)			
Median (IQR)	22.5 (11.0–44.6)	26.8 (11.7–49.6)	0.34
DiMeIQx (ng/d)			
Median (IQR)	1.0 (0.3–5.2)	1.3 (0.4–2.8)	0.71
Total energy intake (kcal/d)			
Median (IQR)	2,242 (1,780–2,811)	2,240 (1,746–2,820)	0.71

Abbreviation: IQR, interquartile range.
*P values derived from t test or χ^2 test.
†Aggressive disease is characterized as Gleason score ≥7 or stage ≥III.

Results

A summary of the SNPs, in chromosomal order, within each gene or gene region that were evaluated in this study is given in Supplementary Appendix A. Among the control group, 14 genotypes deviated from Hardy-Weinberg proportions (P < 0.05) but no significant deviations were observed among the SNPs presented in the results tables. Cases were slightly older than controls; however, this difference was not statistically significant (Table 1).

Table 2 presents the ORs and 95% CIs for the main effect of HCAs and risk of prostate cancer risk in the current analysis and in

Table 2. ORs and 95% CIs for the main effect of HCAs and risk of prostate cancer risk (N = 1,126 cases and 1,127 controls)

Cross et al. quintiles*			Interaction categories			
	Cases	OR (95% CI)		Cases	Controls	OR (95% CI)
PhIP (ng/d)			PhIP (ng/d)			
0.0–25.5	280	Reference	—	—	—	—
>25.5–56.1	280	1.05 (0.88–1.24)	—	—	—	—
>56.1–112.7	272	1.07 (0.89–1.27)	0.0–61.6	478	451	Reference
>112.7–269.2	247	1.04 (0.87–1.25)	>61.6–269.0	419	450	1.00 (0.81–1.22)
>269.2	259	1.22 (1.01–1.48)	>269.0	230	225	1.11 (0.86–1.44)
MeIQx (ng/d)			MeIQx (ng/d)			
0.0–9.8	299	Reference	—	—	—	—
>9.8–19.4	300	1.04 (0.88–1.24)	—	—	—	—
>19.4–33.1	260	1.00 (0.82–1.22)	0.0–20.8	532	467	Reference
>33.1–59.4	242	0.97 (0.78–1.21)	20.8–58.7	403	433	0.87 (0.69–1.09)
>59.4	237	0.97 (0.76–1.24)	>58.7	192	226	0.80 (0.57–1.12)
DiMeIQx (ng/d)			DiMeIQx (ng/d)			
0.0–0.2	277	Reference	—	—	—	—
>0.2–0.7	310	1.14 (0.96–1.35)	—	—	—	—
>0.7–1.6	376	1.04 (0.86–1.25)	0.0–0.8	500	451	Reference
>1.6–3.4	242	0.94 (0.76–1.16)	>0.8–3.5	431	450	0.93 (0.74–1.16)
>3.4	233	0.98 (0.77–1.25)	>3.5	196	225	0.91 (0.65–1.27)

NOTE: Adjusted for age at selection, number of screening exams, study center, family history of prostate cancer, diabetes, smoking status, physical activity, aspirin use, body mass index, total energy intake, dietary lycopene, supplemental vitamin E, and other HCAs.
*Adapted from Cross et al. (11).

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-2447

the previous analysis of the PLCO cohort. Consistent with the previous analysis, there was an elevated risk of prostate cancer in the top quintile of PhIP intake when compared with the reference interaction category (OR, 1.11; 95% CI, 0.86–1.44), although in this modified population, the risk did not reach statistical significance. The current analysis has ~200 fewer prostate cancer cases than previously reported and is oversampled for advanced disease, some of which were not included in the previous cohort analysis and thus likely explains the few differences in risk estimates.

Fifteen interactions with $P \leq 0.15$ were observed between HCAs and several phase I/II enzyme SNPs, including those in the *CYP1B1, GSTP1, GSTM3*, and *UGT1A* gene and gene regions (Table 3). Interaction *P* values between MeIQx and DiMeIQx and the *GSTM3* polymorphism, rs11102001, were particularly small ($P_{interaction}$ = 0.001 for both HCAs) and indicated that the effect of HCA intake on the risk of prostate cancer may differ by genotype. The FDR-adjusted *P* values for the interactions involving MeIQx-rs11102001 and DiMeIQx-rs11102001 were both 0.12 and, thus, statistically significant at the FDR = 0.20 level. The GSTM3 SNP, rs11102001, also seemed to modify the association between PhIP and prostate cancer risk ($P_{interaction}$ = 0.04). Similarly, the *GSTP1* nonsynonymous SNP, rs1695, seemed to modify the association between PhIP and MeIQx intake and prostate cancer ($P_{interaction}$ = 0.03 for both HCAs). Neither these nor other interactions were deemed significant after adjustment using the FDR method (FDR > 0.20).

Associations between HCA intake and prostate cancer stratified by *GSTM3* and *GSTP1* genotypes are presented in Table 4. Among individuals carrying the *AG* or *AA* genotype (rs11102001), the risk of prostate cancer for those with high intake of MeIQx and DiMeIQx was increased compared with those with low intake [OR, 1.7 (95% CI, 0.8–3.6; $P_{interaction}$ = 0.001) and OR, 2.3 (95% CI, 1.2–4.7; $P_{interaction}$ = 0.001), respectively]. Among individuals carrying the *GG* genotype, risk of prostate cancer was decreased compared with those with low MeIQx and DiMeIQx intake [OR, 0.6 (95% CI, 0.5–0.8) and OR, 0.7 (95% CI, 0.5–0.8), respectively]. Increased intake of PhIP or MeIQx was inversely associated with risk of prostate cancer among individuals with *AA* or *AG* (rs1695) genotypes, but a positive association was observed among individuals with the *GG* genotype [high versus low PhIP: OR, 1.5 (95% CI, 0.7–3.1) and high versus low MeIQx: OR, 1.5 (95% CI, 0.7–3.3)]. Similar findings were observed among homozygous variant genotypes GG for rs2274536 (*GSTM3*), TT for rs18887546 (*GSTM3*), and GG for rs6591256 (*GSTP1*) in which high HCA intake was associated with increased risk of prostate cancer compared with low intake, whereas wild-type variants were associated with decreased risk. Thus, the effect of HCAs on prostate cancer seems to depend on genotype.

The associations between joint categories of HCA intake and *GSTM3* and *GSTP1* genotypes and prostate cancer risk are summarized in Supplementary Appendix B. The patterns of joint effects were similar to the findings from the analyses stratified by genotype, with an increased association with high HCA intake observed among homozygous variant genotypes; however, the magnitude of these effects (OR) tended to be in the range of 1.1 to 1.5.

GSTP1 and *GSTM3* haplotype interactions were also evaluated given that several SNPs in these genes showed interesting interactions. PhIP and MeIQx interactions with *GSTP1* haplotypes were associated with prostate cancer ($P_{interaction}$ = 0.06 and $P_{interaction}$ = 0.02, respectively; data not shown). These associations were completely driven by the rs1695 polymorphism, in which haplotypes that included the variant allele at rs1695 resulted in increased risk of prostate cancer. Important associations for

Table 3. HCA-SNP interaction *P* values by HCA for all interactions at $P \leq 0.15$, and risk of prostate cancer

Gene/gene region	dbSNP identifier	Location	Amino acid change	Main effect *P* value$_{(1\ df)}$	$P_{interaction}$*
PhIP					
GSTP1	rs6591256	−1415		0.58	0.11
GSTP1	rs947894 (rs1695)	Ex5-24	I105V	0.78	0.03
GSTM3 (downstream)†	rs2274536	IVS15+40		0.31	0.11
GSTM3 (downstream)†	rs1887546	IVS13+96		0.35	0.13
GSTM3 (downstream)†	rs11102001	Ex12-53	P356S	0.69	0.04
UGT1A locus	rs4148325/rs6742078‡	IVS1-2371		0.21	0.05
UGT1A locus	rs6717546	*913		0.13	0.12
UGT1A locus	rs2018985	IVS1-26820		0.38	0.14
MeIQx					
CYP1B1	rs10916	Ex3+1284		0.27	0.04
GSTP1	rs947894 (rs1695)	Ex5-24	I105V	0.78	0.03
GSTM3 (downstream)†	rs11102001	Ex12-53	P356S	0.69	0.001§
DiMeIQx					
GSTM3 (downstream)†	rs11102001	Ex12-53	P356S	0.69	0.001§
GSTM3 (downstream)†	rs2274536	IVS15+40		0.31	0.08
UGT1A locus	rs10176426	IVS1+52707		0.53	0.01
UGT1A locus (downstream)	rs4663335	IVS3+592		0.92	0.05

*P value is for a test for interaction using the likelihood ratio test comparing models with and without the cross-product of SNP for the given gene and categories of HCA intake.

†SNP located within 10 kb downstream of *EPS8L3*.

‡r^2 = 1.

§Significant at the FDR = 0.20 level.

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-2447

Table 4. Association between HCA intake and prostate cancer stratified by *GSTM3* and *GSTP1* genotypes for which HCA-SNP interactions were $P < 0.15$

Exposure	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
GSTM3						
rs11102001		GG		AG + AA		
PhIP						
Low	414/370	1.0	47/67	1.0		
Medium	246/390	0.8 (0.7–1.0)	61/53	1.4 (0.8–2.5)		
High	189/191	0.9 (0.7–1.2)	29/27	1.6 (0.9–3.1)		
P_{trend}		0.23		0.14		
$P_{interaction}$						0.04
MeIQx						
Low	458/377	1.0	50/73	1.0		
Medium	334/369	0.8 (0.6–0.9)	55/54	1.4 (0.8–2.5)		
High	157/205	0.6 (0.5–0.8)	29/20	1.7 (0.8–3.6)		
P_{trend}		0.01		0.14		
$P_{interaction}$						0.001*
DiMeIQx						
Low	435/363	1.0	51/74	1.0		
Medium	356/382	0.8 (0.7–1.0)	54/55	1.4 (0.8–2.5)		
High	158/206	0.7 (0.5–0.8)	32/18	2.3 (1.2–4.7)		
P_{trend}		0.01		0.02		
$P_{interaction}$						0.001*
rs2274536		AA		AG		GG
PhIP						
Low	231/213	1.0	197/182	1.0	33/42	1.0
Medium	163/209	0.7 (0.5–0.9)	203/193	1.0 (0.8–1.4)	41/41	1.6 (0.8–3.1)
High	95/109	0.8 (0.6–1.1)	100/89	1.2 (0.8–1.7)	23/20	1.9 (0.8–4.7)
P_{trend}		0.06		0.48		0.11
$P_{interaction}$						0.11
DiMeIQx						
Low	236/209	1.0	210/184	1.0	40/44	1.0
Medium	182/213	0.7 (0.6–1.0)	194/185	1.0 (0.7–1.3)	34/39	1.1 (0.5–2.1)
High	71/109	0.5 (0.4–0.8)	96/95	0.9 (0.7–1.3)	23/20	1.7 (0.8–4.0)
P_{trend}		0.01		0.67		0.24
$P_{interaction}$						0.08
rs18887546		GG		GT		TT
PhIP						
Low	231/213	1.0	197/182	1.0	33/42	1.0
Medium	165/211	0.7 (0.5–0.9)	202/191	1.1 (0.8–1.4)	39/41	1.5 (0.8–3.0)
High	96/110	0.8 (0.6–1.1)	99/88	1.2 (0.8–1.7)	23/20	2.0 (0.8–4.9)
P_{trend}		0.06		0.47		0.10
$P_{interaction}$						0.13
GSTP1						
rs1695		AA		AG		GG
PhIP						
Low	205/194	1.0	201/185	1.0	55/58	1.0
Medium	192/184	1.0 (0.8–1.4)	160/211	0.7 (0.5–0.9)	55/48	1.4 (0.8–2.6)
High	85/109	0.8 (0.6–1.1)	108/90	1.1 (0.7–1.5)	24/19	1.5 (0.7–3.1)
P_{trend}		0.25		0.83		0.21
$P_{interaction}$						0.03
MeIQx						
Low	237/216	1.0	209/179	1.0	62/55	1.0
Medium	176/168	1.0 (0.8–1.4)	170/203	0.7 (0.5–1.0)	45/52	0.8 (0.5–1.5)
High	69/103	0.6 (0.5–1.0)	90/104	0.7 (0.5–1.0)	27/18	1.5 (0.7–3.3)
P_{trend}		0.06		0.82		0.50
$P_{interaction}$						0.03
rs6591256		AA		AG		GG
PhIP						
Low	155/153	1.0	230/205	1.0	76/79	1.0

(*Continued on the following page*)

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-2447

Table 4. Association between HCA intake and prostate cancer stratified by *GSTM3* and *GSTP1* genotypes for which HCA-SNP interactions were $P < 0.15$ (Cont'd)

Exposure	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
Medium	143/144	1.0 (0.7–1.3)	183/219	0.8 (0.6–1.0)	80/79	1.1 (0.7–1.7)
High	68/81	0.8 (0.6–1.2)	113/113	0.9 (0.6–1.3)	36/24	1.6 (0.8–3.0)
P_{trend}		0.06		0.48		0.11
$P_{interaction}$						0.11

NOTE: Adjusted for age at selection, study center, and total energy intake.
*Significant at the FDR = 0.20 level.

GSTM3 haplotype-HCA interactions were also present: GSTM3-MeIQx, $P_{interaction} = 0.04$; GSTM3-DiMeIQx, $P_{interaction} = 0.08$ (data not shown). Haplotypes that carried the variant alleles in rs7483, rs2274536, rs1887546, and rs11102001 were observed to increase risk; however, these haplotype frequencies were extremely rare (<0.01%), resulting in unstable estimates and are therefore not shown.

Discussion

Our findings offer the first comprehensive analysis of the interaction between HCA intake, genetic polymorphisms in xenobiotic metabolizing genes that are known to metabolize these compounds, and prostate cancer risk. We observed that the effect of HCA intake on the risk of prostate cancer differs by *GSTM3* and *GSTP1* genotypes in particular; interactions with the *EPS8L3* Pro356Ser polymorphism (rs11102001) just downstream of the *GSTM3* locus were statistically significant at the FDR = 0.20 level and the *GSTP1* Ile105Val polymorphism (rs1695) also seemed to modify risk.

We observed suggestive HCA interactions with three SNPs located in the *GSTM3* region (rs2274536, rs1887546, and rs11102001). All three of these SNPs are located within the 10-kb downstream margin of *GSTM3* and are located in another gene called *EPS8L3* (epidermal growth factor receptor pathway substrate 8–like 3). This gene, and other members of the *EPS8* family (*EPS8L1* and *EPS8L2*), encodes proteins that are responsible for Ras to Rac signaling leading to actin remodeling or cytoskeletal integrity (28–30). The Ras signaling pathway regulates normal cell proliferation. Ras and Ras-related proteins are often deregulated in cancers, leading to increased invasion, metastasis, and decreased apoptosis (31). Ras activation is a component of the signaling pathways for virtually all the receptors shown to be up-regulated in advanced prostate cancer (32). The polymorphic site at codon 356 in exon 12 (rs11102001), in which a guanine-to-adenosine (G-A) transition occurs, causes a proline-to-serine substitution and had a minor allele frequency of 7% among controls in our population, which is consistent with the minor allele frequency observed in HapMap for Caucasians (7%). Although no literature describes any loss of activity associated with this polymorphism or that this amino acid substitution is likely to be damaging (PolyPhen[5] prediction score, 0.286), carriers of the variant allele (*A*) with high MeIQx and DiMeIQx intake were at higher risk for prostate cancer compared with those with low intake. A recent study showed that PhIP stimulates cellular signaling pathways resulting in increased growth and cell migration in human mammary epithelial cell lines (33). Thus, increased HCA exposure could similarly act as a promoter of malignant transformation by increasing mitogenic signaling.

Alternatively, the mechanism of action responsible for the observed effect might be linked to xenobiotic metabolism pathways. *GSTM3* is expressed in prostate tissues (34, 35) and acts to detoxify active HCA metabolites by conjugation with glutathione (15). Altered expression of the enzyme could lead to differential clearance of activated HCA metabolites resulting in an accumulation of DNA-damaging species, which could increase the risk for carcinogenesis at this site. The three *EPS8L3* SNPs are located just downstream of *GSTM3* and show strong linkage disequilibrium with variants in the *GSTM3* locus; therefore, it is possible that these SNPs are surrogate markers of other variants in the *GSTM3* locus that were not genotyped. Alternatively, these downstream variants could alter *GSTM3* expression. After adjustment using the FDR method, the MeIQx-rs11102001 and DiMeIQx-rs11102001 interactions seem to be the most interesting findings, suggesting a real modification of prostate cancer risk. Thus, further examination of variants in this region is warranted.

HCA intake was estimated from a meat-cooking FFQ module, which included questions about meat type, cooking method, and cooking time, or "doneness" level, used in conjunction with a HCA database. HCA formation in meat generally increases with temperature and doneness level. PhIP is the most abundant HCA, whereas MeIQx and DiMeIQx are the least abundant; 2-amino-3methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) are not typically detected in meat samples (36). The questionnaire and procedure for estimating HCA intake has been validated and found to be an acceptable method for HCA exposure assessment (37).

We observed an effect for DiMeIQx and MeIQx in the presence of *GSTM3* variants. Although DiMeIQx is consumed at a low level with respect to other HCAs in the diet, DiMeIQx and MeIQx are observed to be more potent mutagens than PhIP (38), and thus their effect on carcinogenesis may be greater. There is little information in the literature with respect to the activity of GST enzymes in the detoxification of DiMeIQx and MeIQx. Because the formation of PhIP is the highest, the pathway for detoxification of this compound is better described with respect to several phase II enzymes (15). Information on DiMeIQx is lacking with respect to detoxification pathways and carcinogenicity data, although it is known to be mutagenic in bacterial assays. The continued

[5] http://genetics.bwh.harvard.edu/pph/

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-2447

evaluation of DiMeIQx and MeIQx is needed as more studies of HCAs and cancer risk implicate these more potent HCAs.

We also observed lesser effects for HCA interactions with one promoter polymorphism (rs6591256, position −1415) and one nonsynonymous polymorphism (rs1695, isoleucine-to-valine substitution) in *GSTP1*. *GSTP1* is the major GST identified in benign prostate hyperplasia tissue samples relative to other GST enzymes (35, 39). In human prostate tissue, however, expression of this enzyme is silenced via hypermethylation of the promoter region (40), which occurs in ~90% of prostate adenocarcinomas (40, 41), suggesting that alteration of *GSTP1* activity is related to prostate carcinogenesis. The polymorphic site at codon 105 in exon 5, in which an adenosine-to-guanine (A-G) transition causes an isoleucine-to-valine substitution (rs1695), has also been extensively described (42). The valine substitution results in lower enzyme activity (43, 44) for certain substrates and thus may impair the detoxification of carcinogens. Although these interactions were not statistically significant, consideration of both genetic and environmental exposures may offer additional insight into our understanding given the known biological effects of altered *GSTP1* expression in prostate carcinogenesis.

Although we observed some modifying effects in SNPs in the *UGT1A* locus and in *CYP1B1*, the magnitude of these findings was not as large as those for *GSTM3* and *GSTP1*. We also did not observe a strong modifying effect for SNPs in *CYP1A1, CYP1A2, GSTA1, GSTM1, NAT1, NAT2, SULT1A1*, and *SULT1A2*. The lack of findings in these genes does not mean that they do not play a role in altering risk via differential HCA metabolism. Variants in *CYP1A2* and in the *NAT*s have been well described in HCA metabolism and often implicated to potentially alter cancer risk (45, 46). *CYP1A2* is the principal hepatic CYP involved in the bioactivation of HCAs to their *N*-hydroxy metabolites, and *NAT*s are responsible for further bioactivation of *N*-hydroxy-HCA metabolites to genotoxic *N*-acetoxy-HCA esters (27). It is possible that we did not find an association for these genes because the bioactivation process is less important than the detoxification processes performed by the GSTs or the expression of these enzymes in the target tissue, the prostate, is lower. However, it is also possible that important SNPs in these genes and others were not genotyped or captured by our tagSNPs or that the power to detect these associations was low.

The strengths of our study included a relatively large sample, a detailed assessment of dietary HCA intake, and a comprehensive characterization of the genes involved in HCA metabolism. Given that this study was nested within the screening arm of the PLCO trial, selection and surveillance bias is minimal because both cases and controls had an equal opportunity to be detected with prostate cancer. Although this is the largest study to evaluate the interaction between dietary HCA intake and genetic variants in XME genes and risk for prostate cancer, the presence of low-frequency variants limits the power to detect significant interactions. Thus, we have attempted to identify interesting associations that need to be followed up in future studies. Despite the potential limitation in power, it was important to characterize the top HCA intake category using the highest quintile of exposure based on previous positive associations with PhIP and prostate cancer only in the top quintile of intake. This study is composed only of non-Hispanic Caucasian men, which limits the generalizability of our findings to other race/ethnic groups with different genetic compositions; however, as a result of the racial homogeneity, population stratification is unlikely to be a significant source of bias in this study (47).

Data from genome-wide association studies have yielded novel and interesting insights into genetic risks for prostate cancer. These types of studies, however, do not take into consideration the complex interaction between genetic variants and environmental exposures. In conclusion, variants in two genes known to detoxify HCAs, *GSTM3/EPS8L3* and *GSTP1*, modify the association between dietary HCA intake and prostate cancer. Despite the fact that genome-wide scans have not identified these genes as important prostate cancer risk factors in main effect studies, additional studies with more power should continue to evaluate these genes in relation to environmental interactions and prostate cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

Received 6/26/2008; revised 10/14/2008; accepted 12/15/2008; published OnlineFirst 02/17/2009.

Grant support: Intramural Research Program of the NIH (National Cancer Institute, Division of Cancer Epidemiology and Genetics) and grant TU2 CA105666 from National Cancer Institute.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

1. Stuart GR, Holcroft J, de Boer JG, Glickman BW. Prostate mutations in rats induced by the suspected human carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. Cancer Res 2000;60:266–8.
2. Shirai T, Sano M, Tamano S, et al. The prostate: a target for carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) derived from cooked foods. Cancer Res 1997;57:195–8.
3. Cui L, Takahashi S, Tada M, et al. Immunohistochemical detection of carcinogen-DNA adducts in normal human prostate tissues transplanted into the subcutis of athymic nude mice: results with 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 3,2′-dimethyl-4-aminobiphenyl (DMAB) and relation to cytochrome *P*450s and *N*-acetyltransferase activity. Jpn J Cancer Res 2000;91:52–8.
4. Martin FL, Cole KJ, Muir GH, et al. Primary cultures of prostate cells and their ability to activate carcinogens. Prostate Cancer Prostatic Dis 2002;5:96–104.
5. Wang CY, biec-Rychter M, Schut HA, et al. *N*-Acetyltransferase expression and DNA binding of *N*-hydroxyheterocyclic amines in human prostate epithelium. Carcinogenesis 1999;20:1591–5.
6. Tang D, Liu JJ, Bock CH, et al. Racial differences in clinical and pathological associations with PhIP-DNA adducts in prostate. Int J Cancer 2007;121:1319–24.
7. Tang D, Liu JJ, Rundle A, et al. Grilled meat consumption and PhIP-DNA adducts in prostate carcinogenesis. Cancer Epidemiol Biomarkers Prev 2007;16:803–8.
8. U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program. Report on Carcinogens. 11th ed. 2005.
9. Archer CL, Morse P, Jones RF, Shirai T, Haas GP, Wang CY. Carcinogenicity of the *N*-hydroxy derivative of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, 2-amino-3,8-dimethyl-imidazo[4,5-*f*]quinoxaline and 3,2′-dimethyl-4-aminobiphenyl in the rat. Cancer Lett 2000;155:55–60.
10. Poirier LA, Weisburger EK. Selection of carcinogens and related compounds tested for mutagenic activity. J Natl Cancer Inst 1979;62:833–40.
11. Cross AJ, Peters U, Kirsh VA, et al. A prospective study of meat and meat mutagens and prostate cancer risk. Cancer Res 2005;65:11779–84.
12. Koutros S, Cross AJ, Sandler DP, et al. Meat and meat mutagens and risk of prostate cancer in the agricultural health study. Cancer Epidemiol Biomarkers Prev 2008;17:80–7.
13. Norrish AE, Ferguson LR, Knize MG, Felton JS, Sharpe SJ, Jackson RT. Heterocyclic amine content of cooked meat and risk of prostate cancer. J Natl Cancer Inst 1999;91:2038–44.
14. Rovito PM, Jr., Morse PD, Spinek K, et al. Heterocyclic amines and genotype of *N*-acetyltransferases as risk

Published OnlineFirst February 17, 2009; DOI: 10.1158/0008-5472.CAN-08-2447

factors for prostate cancer. Prostate Cancer Prostatic Dis 2005;8:69–74.
15. Turesky RJ. The role of genetic polymorphisms in metabolism of carcinogenic heterocyclic aromatic amines. Curr Drug Metab 2004;5:169–80.
16. Nock NL, Liu X, Cicek MS, et al. Polymorphisms in polycyclic aromatic hydrocarbon metabolism and conjugation genes, interactions with smoking and prostate cancer risk. Cancer Epidemiol Biomarkers Prev 2006;15:756–61.
17. Nowell S, Ratnasinghe DL, Ambrosone CB, et al. Association of SULT1A1 phenotype and genotype with prostate cancer risk in African-Americans and Caucasians. Cancer Epidemiol Biomarkers Prev 2004;13:270–6.
18. Ntais C, Polycarpou A, Tsatsoulis A. Molecular epidemiology of prostate cancer: androgens and polymorphisms in androgen-related genes. Eur J Endocrinol 2003;149:469–77.
19. Steiner M, Bastian M, Schulz WA, et al. Phenol sulphotransferase SULT1A1 polymorphism in prostate cancer: lack of association. Arch Toxicol 2000;74:222–5.
20. Prorok PC, Andriole GL, Bresalier RS, et al. Design of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Control Clin Trials 2000;21:273–309S.
21. Hayes RB, Reding D, Kopp W, et al. Etiologic and early marker studies in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. Control Clin Trials 2000;21:349–55S.
22. Yeager M, Orr N, Hayes RB, et al. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. Nat Genet 2007;39:645–9.
23. Sinha R, Cross A, Curtin J, et al. Development of a food frequency questionnaire module and databases for compounds in cooked and processed meats. Mol Nutr Food Res 2005;49:648–55.
24. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet 2004;74:106–20.
25. Schut HA, Snyderwine EG. DNA adducts of heterocyclic amine food mutagens: implications for mutagenesis and carcinogenesis. Carcinogenesis 1999;20:353–68.
26. Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 1995;12:921–7.
27. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B Methodol 1995;57:289–300.
28. Offenhauser N, Borgonovo A, Disanza A, et al. The eps8 family of proteins links growth factor stimulation to actin reorganization generating functional redundancy in the Ras/Rac pathway. Mol Biol Cell 2004;15:91–8.
29. Tocchetti A, Confalonieri S, Scita G, Di Fiore PP, Betsholtz C. *In silico* analysis of the EPS8 gene family: genomic organization, expression profile, and protein structure. Genomics 2003;81:234–44.
30. Welsch T, Endlich K, Giese T, Buchler MW, Schmidt J. Eps8 is increased in pancreatic cancer and required for dynamic actin-based cell protrusions and intercellular cytoskeletal organization. Cancer Lett 2007;255:205–18.
31. Bos JL. ras oncogenes in human cancer: a review. Cancer Res 1989;49:4682–9.
32. Weber MJ, Gioeli D. Ras signaling in prostate cancer progression. J Cell Biochem 2004;91:13–25.
33. Creton SK, Zhu H, Gooderham NJ. The cooked meat carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine activates the extracellular signal regulated kinase mitogen-activated protein kinase pathway. Cancer Res 2007;67:11455–62.
34. Bostwick DG, Meiers I, Shanks JH. Glutathione *S*-transferase: differential expression of α, μ, and π isoenzymes in benign prostate, prostatic intraepithelial neoplasia, and prostatic adenocarcinoma. Hum Pathol 2007;38:1394–401.
35. Di Paolo OA, Teitel CH, Nowell S, Coles BF, Kadlubar FF. Expression of cytochromes *P*450 and glutathione *S*-transferases in human prostate, and the potential for activation of heterocyclic amine carcinogens via acetyl-coA-, PAPS- and ATP-dependent pathways. Int J Cancer 2005;117:8–13.
36. Sinha R. An epidemiologic approach to studying heterocyclic amines. Mutat Res 2002;506–7:197–204.
37. Cantwell M, Mittl B, Curtin J, et al. Relative validity of a food frequency questionnaire with a meat-cooking and heterocyclic amine module. Cancer Epidemiol Biomarkers Prev 2004;13:293–8.
38. Felton JS, Knize MG. Occurrence, identification, and bacterial mutagenicity of heterocyclic amines in cooked food. Mutat Res 1991;259:205–17.
39. Di Ilio C, Aceto A, Bucciarelli T, et al. Glutathione transferase isoenzymes from human prostate. Biochem J 1990;271:481–5.
40. Brooks JD, Weinstein M, Lin X, et al. CG island methylation changes near the GSTP1 gene in prostatic intraepithelial neoplasia. Cancer Epidemiol Biomarkers Prev 1998;7:531–6.
41. Millar DS, Ow KK, Paul CL, Russell PJ, Molloy PL, Clark SJ. Detailed methylation analysis of the glutathione *S*-transferase pi (GSTP1) gene in prostate cancer. Oncogene 1999;18:1313–24.
42. Zimniak P, Nanduri B, Pikula S, et al. Naturally occurring human glutathione *S*-transferase GSTP1-1 isoforms with isoleucine and valine in position 104 differ in enzymic properties. Eur J Biochem 1994;224:893–9.
43. Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J. Molecular cloning, characterization, and expression in *Escherichia coli* of full-length cDNAs of three human glutathione *S*-transferase π gene variants. Evidence for differential catalytic activity of the encoded proteins. J Biol Chem 1997;272:10004–12.
44. Hu X, Xia H, Srivastava SK, et al. Catalytic efficiencies of allelic variants of human glutathione *S*-transferase P1-1 toward carcinogenic anti-diol epoxides of benzo[*c*]phenanthrene and benzo[*g*]chrysene. Cancer Res 1998;58:5340–3.
45. Ishibe N, Sinha R, Hein DW, et al. Genetic polymorphisms in heterocyclic amine metabolism and risk of colorectal adenomas. Pharmacogenetics 2002;12:145–50.
46. Lilla C, Verla-Tebit E, Risch A, et al. Effect of NAT1 and NAT2 genetic polymorphisms on colorectal cancer risk associated with exposure to tobacco smoke and meat consumption. Cancer Epidemiol Biomarkers Prev 2006;15:99–107.
47. Wacholder S, Rothman N, Caporaso N. Counterpoint: bias from population stratification is not a major threat to the validity of conclusions from epidemiological studies of common polymorphisms and cancer. Cancer Epidemiol Biomarkers Prev 2002;11:513–20.

Cancer Research
The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

AACR American Association for Cancer Research

Xenobiotic Metabolizing Gene Variants, Dietary Heterocyclic Amine Intake, and Risk of Prostate Cancer

Stella Koutros, Sonja I. Berndt, Rashmi Sinha, et al.

Cancer Res 2009;69:1877-1884. Published OnlineFirst February 17, 2009.